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ESSENTIALITY AND FUNCTION OF NICKEL[1]

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Until recently, only indirect evidence has been available to suggest that nickel has an essential physiological role in animals. The data have been obtained from pharmacologic, toxicologic, and *in vitro* biochemical studies. Other evidence has been obtained from analyses which indicate that nickel is consistently present in some biological materials, and which show changes in tissue nickel distribution, or concentrations, in some pathological conditions. Since this evidence has been recently reviewed[2], it will not be discussed here. Instead, evidence which directly shows that nickel has an essential function, or functions, will be presented.

ESSENTIALITY OF NICKEL

Nickel is an essential nutrient for animals. The following criteria for essentiality have been met:

(1) It has a low molecular weight and is a transition element which forms chelates, and thus is chemically suitable for biological functions.

(2) It is ubiquitous on the earth's crust and in sea water; therefore, it has been generally available to plants and animals during their evolution.

(3) It is present in plants and animals.

(4) It is nontoxic to animals orally except in astringent doses.

(5) Homeostatic mechanisms are implied by serum levels, excretion rates, and lack of excessive accumulation.

1. Mention of a proprietary product does not necessarily imply endorsement by the United States Department of Agriculture.

2. F. H. Nielsen and D. A. Ollerich. 1973. Nickel: A new essential trace element. Presented at the 57th Annual Federation of American Societies for Experimental Biology Meeting, Atlantic City, N.J. Submitted for publication in *Federation Proceedings.*

(6) Its deficiency reproducibly results in an impairment of a function, or functions, from optimal to suboptimal.

The first four of these criteria have been adequately reviewed (1–3). The last two criteria have been studied more extensively recently, and therefore will be discussed in more detail.

Nickel is poorly absorbed from ordinary diets and is excreted mostly in the feces. This is apparent from studies with dogs (4) and man (5, 6). Nodiya (5) performed nickel balance studies on 10 Russian males, age 17 years, who were ingesting a mean of 289 ± 23 μg Ni/day (range, 251–309). He found that fecal excretion of nickel averaged 258 ± 23 μg/day (range, 219–278). Horak and Sunderman (6) measured fecal nickel excretion in 10 healthy subjects (age 22–65; four males, six females) who ingested varied diets which were prepared in their own homes. They found that the fecal excretion of nickel averaged 258 ± 126 μg/day (range, 80–540). Most of this nickel was probably that which was not absorbed; however, nickel has been found in the bile of rats (7), so some fecal nickel may come from the bile. Urinary nickel excretion is 10–100 times less than fecal excretion. For example, Perry and Perry (8) found that 24 healthy adults excreted an average of 20 μg/day in the urine, whereas Sunderman (9) noted a mean daily urinary excretion of 19.8 μg in 17 normal adults. In another study, Nomoto and Sunderman (10) found that 26 healthy subjects had a mean daily urinary excretion of 2.4 μg/day. In addition to the feces and urine, there is evidence that sweat may be important in nickel homeostasis. Horak and Sunderman (6) found a mean concentration of 49 μg Ni/liter in sweat collected in plastic bags which encased the arms of five healthy men during sauna bathing. Consolazio *et al.* (11) determined that approximately 8.3 μg Ni is lost in the sweat daily. The concentration of nickel in sweat is approximately 20 times greater than the concentration in serum samples of healthy adults, suggesting that there is active secretion of nickel by the sweat glands. From the previous discussion it is apparent that the absorption of dietary nickel is low, probably in the range of 1–10%, and that fecal excretion is the major route for elimination of ingested nickel from the body. Nickel that is absorbed is apparently lost in the bile, urine, and sweat.

Analyses of tissues indicate that the retained nickel is widely distributed in very low concentrations in the body (2, 12–16). Sunderman, Decsy, and McNeely (17) have found wide variability in the mean concentrations of nickel in sera from several species of animals, but within each species the concentration of serum nickel fell within a relatively narrow range. Examples of mean concentrations and ranges (in μg Ni/liter) include the following: man, 2.6 (1.1–4.6); rats 2.7 (0.9–4.1); chickens, 3.6 (3.3–3.8); rabbits, 9.3 (6.5–14.0). In the serum, nickel exists in three forms: ultrafiltrable, albumin

bound, and as a nickel-metalloprotein (17). The amount of nickel in each compartment varies from species to species and this may be due, in part, to species variation in the affinities of albumin for nickel (18, 19). A metalloprotein designated nickeloplasmin has been isolated from the serum of rabbits and man[3] (20). It is a macroglobulin with an estimated molecular weight of 7.0×10^5 and contains approximately 0.8 g atom Ni/mole. Disc gel and immunoelectrophoresis show that purified nickeloplasmin is an α-1 macroglobulin in rabbit serum and an α-2 macroglobulin in man. Soestbergen and Sunderman (21) have found that the ultrafiltrable nickel in serum does not exist primarily as free Ni^{2+}, but as nickel complexes, and that ultrafiltrable nickel receptors play an important physiological role in nickel homeostasis by serving as diffusible vehicles for the extracellular transport and renal excretion of nickel. Further evidence that nickel as an organic complex is important in metabolism is that Tiffin (22) has found that the translocatable form of nickel in plants appears to be a stable anionic amino acid complex. The bioavailable form of nickel is not known, but since Sunderman[4] has found that nickeloplasmin cannot be labeled by feeding $^{63}NiCl_2$, it is possible that nickel is essential as an organic complex, and that inorganic forms of nickel can fulfill the requirement for nickel only when given in large amounts.

Studies which show a consistent impairment in a physiological function when nickel is deficient in the diet have also recently been successful. Nielsen *et al.* (3, 23, 24) found that feeding a diet containing less than 40 ppb nickel resulted in an apparent nickel-deficiency syndrome in chicks. When compared with controls given a supplement of 3–5 ppm nickel, the deficient chicks showed the following: (1) pigmentation changes in the shank skin; (2) thicker legs with slightly swollen hocks; (3) dermatitis of the shank skin; (4) a less friable liver which may have been related to the fat content; (5) an enhanced accumulation of a tracer dose of ^{63}Ni in liver, bone, and aorta. These findings were observed under conditions which produced suboptimal growth. The abnormalities in leg structure and shank-skin dermatitis were inconsistent. Sunderman *et al.* (25) attempted to confirm Nielsen's findings by feeding a diet containing 44 ppb nickel to chicks raised in a slightly different environment. While they found no gross effects, they did observe ultrastructural changes in the liver. These included dilation of the perimitochondrial rough endoplasmic reticulum in 15–20% of the hepatocytes. In a single experiment with chicks, Leach[5] observed a growth response with nickel. Later attempts

3. F. W. Sunderman, Jr., M. I. Decsy, S. Nomoto, and M. W. Nechay. 1971. Isolation of a nickel-α_2 macroglobulin from human and rabbit serum. *Fed. Proc.* 30: 1274 (abstr.).

4. F. W. Sunderman, Jr. 1973. Personal communication.

5. R. M. Leach, 1973. Personal communication.

to obtain the growth response were unsuccessful. Wellenreiter, Ullrey, and Miller (26) fed a diet containing 80 ppb nickel to reproducing quail and saw no gross symptoms except an inconsistent positive effect of nickel on breast feathering in birds which were relatively arginine deficient but on adequate protein intake. In order to clarify and extend the above observations, improvements were made in the experimental environment used to produce nickel deficiency, and a diet was formulated with a nickel content of 3–4 ppb. With this diet and environment, it has been consistently possible to produce a nickel deficiency in animals.

A major difficulty in the production of nickel deficiency in animals is the preparation of a diet low in nickel. Nickel is ubiquitous. Therefore, the conventional methods of diet preparation using purified proteins, or amino acids, carbohydrates, vitamins, and minerals are not suitable because some contain as much as 20,000 ppb nickel. In order to circumvent this problem, the diet must be prepared from natural feedstuffs low in nickel which contain most of the amino acids, vitamins, and minerals essential for the experimental animal. The diet formulations for the production of nickel deficiency in the chick and rat are presented in Table 1. They are based on dried skim milk, ground corn, and corn oil. The added vitamins include A, D, E, K, niacin, folate, and biotin because skim milk and corn were calculated to provide insufficient amounts. The mineral additions are minimal. The major portion is $CaCo_3$ which is relatively low in nickel (approximately 20 ppb). In order to assure adequate concentrations, Mn, Fe, Zn, Cu, I, and Se are added. The corn must be acid washed to obtain consistently a diet containing 3–4 ppb nickel. In these experiments the control chicks were fed the basal diet supplemented with 3 ppm nickel as $NiCl_2 \cdot 6H_2O$.

A second impediment to the production of nickel deficiency, and also for the production of deficiencies of other trace elements required in very minute amounts, is the environment in which the animals are raised. Significant amounts of trace elements may be present in such sources as caging, feed cups, water bottles, dust in the air, and the skin of the investigator's hands. In order to prevent such contamination, it is necessary to employ techniques similar to those developed by Smith and Schwarz (27).

The experimental animals are raised in a controlled environment such as an all-plastic rigid isolator[6], or a laminar-flow animal rack[7] so that the only materials the animals come into contact with are plastic, and the air entering the system is filtered to remove dust. In order to bring out the effects of nickel deprivation in rats, successive generations were raised. Thus, the

6. Germ Free Laboratories, Inc., Miami, Florida.

7. Carworth, Division of Becton, Dickinson, & Co., New City, New York.

Table 1. Composition of the basal diets[1]

Ingredient	Diet (g/kg)	
	Chick	Rat
Skimmed-milk powder[2]	645.00	645.00
Ground corn, acid-washed[2]	142.50	160.00
Non-nutritive fiber[3]	40.00	40.00
Corn oil[2]	100.00	100.00
Glycine[4]	5.00	
Arginine[4]	25.00	25.00
Choline chloride[2]	0.30	
Vitamins, fat-soluble[5]	0.11	0.16
Vitamin mix[6]	4.59	4.84
Mineral mix[7]	37.50	25.00
	1000.00	1000.00

[1]Diet analyzed 3–4 ppb nickel on an air-dried basis.

[2]Nutritional Biochemicals Corp., Cleveland, Ohio.

[3]Solka Floc, SW-40, Brown Co., Boston, Mass.

[4]General Biochemicals Co., Chagrin Falls, Ohio.

[5]The fat-soluble vitamins were α-tocopherol acetate, 0.01g (chick) or 0.06g (rat), and vitamin D_3 (250 IU/drop), two drops or approximately 0.1g (see footnote 4 in text). These were added to corn oil prior to mixing into diet.

[6]The chick vitamin mix contained (in mg) niacin, 20, folic acid, 1.2, menadione, 0.6, biotin, 0.1, B-carotene, 12, vitamin A palmitate (250,000 IU/g), 4, and glucose, 4552.1. The rat vitamin mix contained (in mg) niacin, 20, folic acid, 1.2, menadione, 0.5, biotin, 0.1, B-carotene, 6, vitamin A palmitate (250,000 IU/g), 4, pyridoxine·HCl, 2, thiamine·HCl, 2, and glucose, 4804.2.

[7]The chick mineral mix contained (in the purest form known available) (in g) $CaCO_3$, 12.5, $MnSO_4 \cdot 5H_2O$, 0.2, iron sponge (dissolved in HCl), 0.055, $CuSO_4$, 0.013, ZnO, 0.025, KI, 0.0005, $(NH_4)_6Mo_7O_{24}$, 0.0035, Na_2SeO_3, 0.006, ground corn (acid-washed), 24.7004. The rat mineral mix contained (in g) $CaCO_3$, 7.5, $MnSO_4 \cdot 5H_2O$, 0.2, iron sponge (dissolved in HCl), 0.05 $CuSO_4$, 0.015, ZnO, 0.015, KI, 0.0005, Na_2SeO_3, 0.006, ground corn (acid-washed), 17.2189.

animals were exposed to deficiency throughout fetal, neonatal, and adult life. Day-old Golden Giant Cockerels[8] were used in the chick experiments. All other experimental materials and methods are described elsewhere[2] (3, 23, 24). Statistical analysis was by the t test (28).

After 3½ weeks, the gross appearances of the deficient and control chicks were similar except for the difference in the pigmentation of their shank skin

8. Jack Frost Chicks, Inc., St. Cloud, Minnesota.

Table 2. Liver and heart analysis of nickel-deficient and supplemented chicks[1]

Group	No. of chicks	O_2 uptake[2] (μliter/hr/mg protein)	Total lipid[3] Liver (%)	Lipid P[3] Liver (mg/g)	Total lipid[3] Heart (mg/g)	Lipid P[3] Heart (mg/g)
			Experiment 1			
Ni def (3 ppb)	12	4.7[4] ± 0.2[5]	6.21[6] ± 0.10			
+ 3 ppm Ni	12	5.5 ± 0.2	5.78 ± 0.11			
			Experiment 2			
Ni def (4 ppb)	11	5.4[7] ± 0.2	6.27[6] ± 0.18	1.327[4] ± 0.016	4.09[6] ± 0.06	0.942[7] ± 0.007
+ 3 ppm Ni	11	6.0 ± 0.2	587 ± 0.05	1.379 ± 0.016	3.85 ± 0.11	0.898 ± 0.010
			Experiment 3			
Ni def (14 ppb)	12	5.5[7] ± 0.1	5.71 ± 0.09	1.318 ± 0.016		
+ 3 ppm	12	5.9 ± 0.1	5.55 ± 0.10	1.335 ± 0.015		

[1]In experiments 1 and 3 the corn was not acid washed.

[2]Using liver homogenates and with α-glycerophosphate as the substrate.

[3]Fresh weight basis.

[4]Significantly different ($P < 0.025$) from +3 ppm Ni group.

[5]± s.e.m.

[6]Significantly different ($P < 0.05$) from +3 ppm Ni group.

[7]Significantly different ($P < 0.10$) from +3 ppm Ni group.

as described previously (3, 23, 24). All chicks weighed 350–400 g. In earlier studies (3, 23, 24), abnormalities in leg structure and a dermatitis were present in the deficient chicks. In the latest experiments, the abnormalities were diminished or inconsistent. It is thought that modification of the diet and improved environmental conditions may have accounted for the decreased incidence or inconsistency of these abnormalities. The other gross sign observed in earlier studies was a decrease in friability of the liver in deficient chicks. This finding was present in chicks raised under the conditions described. Thus, it appears that the change in shank-skin color and the effect on liver consistency are related to nickel status, and that the other gross signs originally described may be less characteristic of nickel deficiency.

In contrast to the gross signs, abnormalities in biochemical indices of metabolism were more consistently found in the nickel-deficient chicks. They included a decreased oxygen uptake by liver homogenates in the presence of α-glycerophosphate, an increase in liver lipids, and a decrease in liver phospholipids (Table 2). On the basis of one experiment, there appears to be an increase in the lipid and phospholipid fraction in the heart.

Ultrastructural abnormalities in the hepatocytes (Figs. 1 and 2) were also a consistent finding. These findings were similar to, though more extensive than, those described by Sunderman *et al.* (25). They included dilation of the cisterns of the rough endoplasmic reticulum and swelling of the mitochondria. The swelling was in the compartment of the matrix and was associated with fragmentation of the cristae. Other ultrastructural changes included a dilation of the perinuclear space and pyknotic nuclei. These ultrastructural abnormalities coupled with the biochemical evidence of deranged metabolism are considered sufficient evidence to indicate that nickel is essential for the chick.

The results of the rat studies are more preliminary, and more experimentation is needed to confirm the results obtained thus far. Reproduction apparently is affected, as seven first-generation nickel-deficient female rats which were mated had a significant number of dead pups (15%) compared with no mortality in the pups of six controls. Nine second-generation nickel-deficient female rats which were mated had a 19% loss of pups. This finding is confounded by the fact that the eight controls had a 10% loss of pups. This was, however, roughly half the loss in the nickel deficient group. The pups of the nickel-deficient dams also weighed slightly less at 4 and 24 (age at weaning) days than those from controls, but the differences were not significant. During the suckling stage, the nickel-deficient pups had generally a less thrifty appearance and were less active. In order to assess this last observation, a Stoelting activity monitor[9] was used to measure the activity of

9. Stoelting Co., Chicago, Illinois.

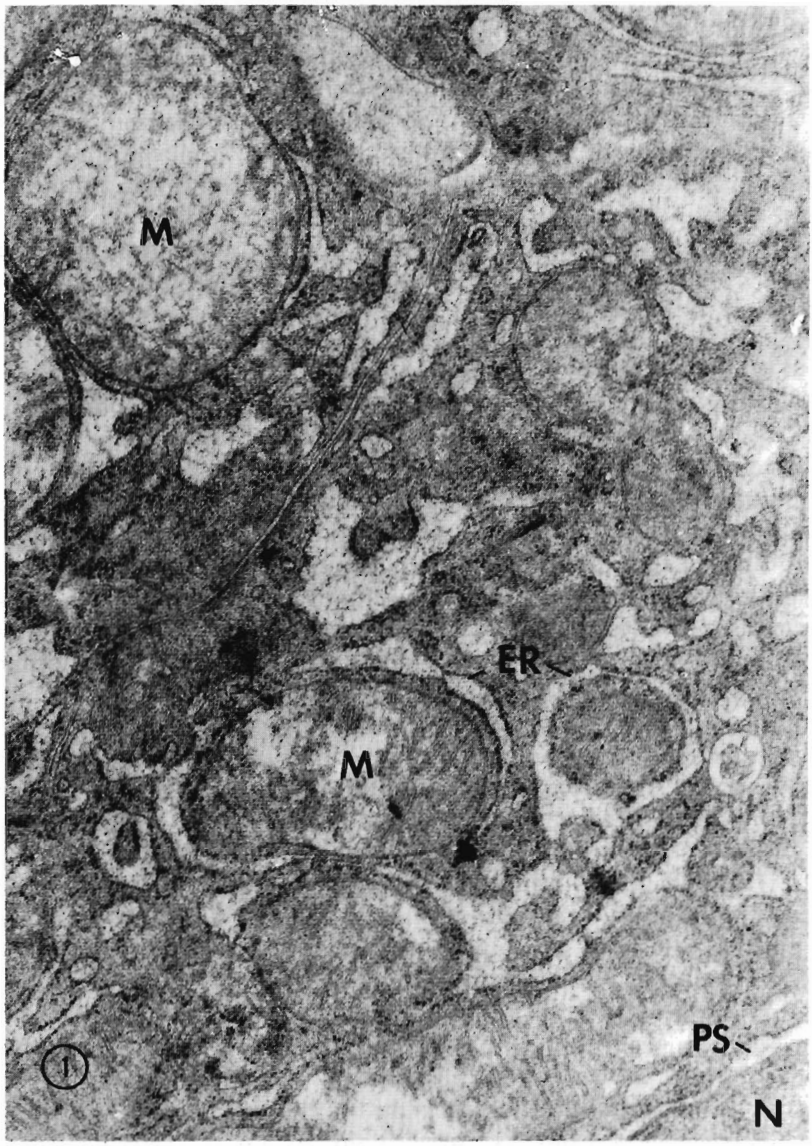

Fig. 1. Hepatic cell from a nickel-deficient chick (4 ppb nickel). Swelling of mitochondria (M) was evident in numerous hepatic cells. The swelling was in the compartment of the matrix and appeared to cause fragmentation of cristae. Note also the dilated cisternae of the rough endoplasmic reticulum (ER) and dilated perinuclear space (PS). Nucleus (N). Uranyl acetate and lead citrate. × 25,500.

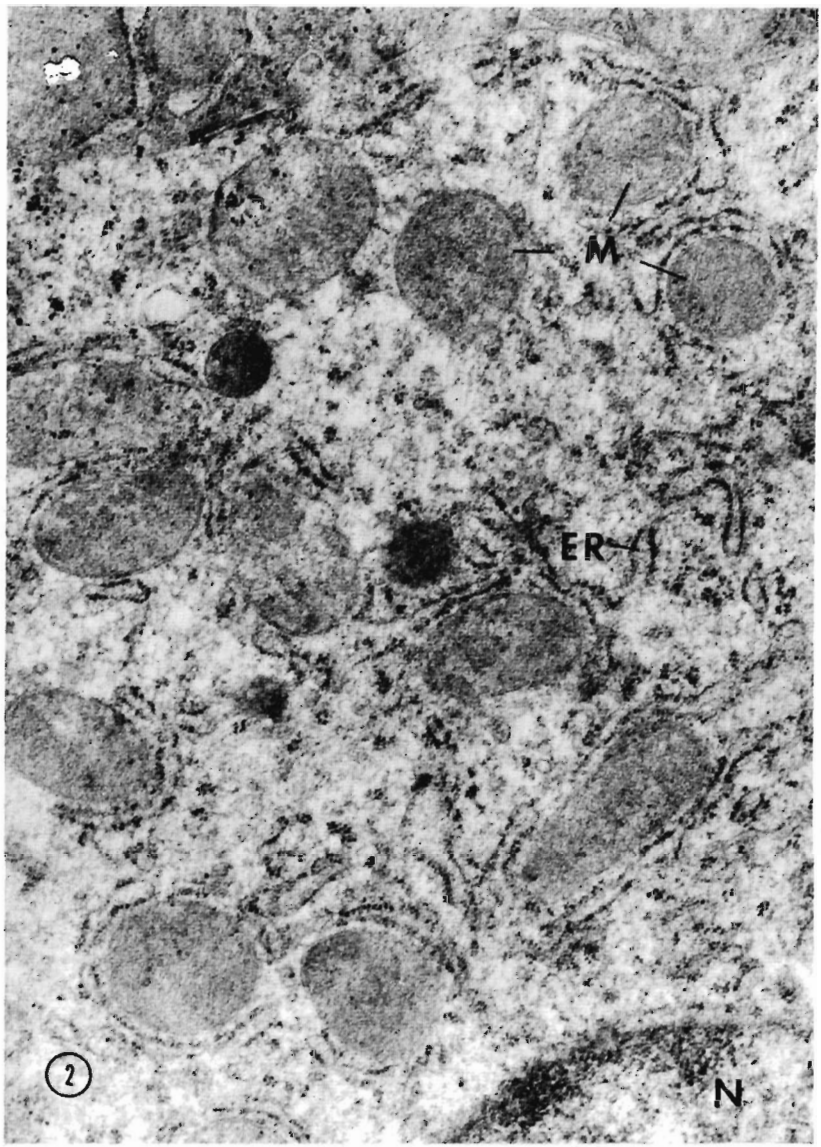

Fig. 2. Hepatic cell from nickel-supplemented chick (3 ppm nickel). Compare mitochondria (M), rough endoplasmic reticulum (ER), and perinuclear space with those in Fig. 1. Nucleus (N). Uranyl acetate and lead citrate. × 25,500.

Table 3. Oxygen uptake of rat-liver homogenates[1]

Group	No. of rats	O_2 uptake (μliter/hr/mg protein)
Ni def (4 ppb)	13	3.20[2] ± 0.08[3]
+ 3 ppm	12	4.17 ± 0.21

[1]α-glycerophosphate as substrate.
[2]Significantly different ($P < 0.001$) from +3 ppm Ni group.
[3]± s.e.m.

matched litters of the same size from both the deficient and control groups in the second and third generation. The results indicate that the nickel-deficient rats are indeed more lethargic.

Twelve each of deficient and control second-generation male rats were killed for measurement of liver oxidative activity. Grossly, it was observed that the livers of the deficient rats had a muddy brown color (compared with a red-brown color of livers of the controls) and a less distinct substrcuture. As in the chick, the deficient rat-liver homogenates showed a reduced oxidative activity in the presence of α-glycerophosphate (Table 3). In addition, preliminary sucrose density gradients of liver post-mitochondrial supernatants were consistent with a decrease in polysomes and an increase in monosomes in the nickel-deficient rat liver.[2]

FUNCTION OF NICKEL

While the above findings indicate that nickel is essential, they provide only meager insights as to its metabolic function. An attractive hypothesis is that nickel has a role in the metabolism or structure of membranes. The swollen mitochondria suggest a possible abnormality in the mitochondrial membrane. The endoplasmic reticulum is a network of membrane-bound cavities. Dilation of the cisterns of the endoplasmic reticulum suggests a possible abnormality in these membranes. Finally, the dilation of the perinuclear space and the presence of pyknotic nuclei in the nickel-deficient liver suggest an abnormality in the nuclear membrane. The level of phospholipids, an integral part of membranes, is also depressed in nickel deficiency. Further credibility for the membrane hypothesis can be obtained from *in vitro* studies with isolated tissues. Nickel can substitute for calcium in certain steps of the excitation-contraction coupling of isolated skeletal muscle (29, 30). Other investigators have found that nickel can also substitute for calcium in the excitation process of the isolated nerve cell (31, 32). It has been postulated

that nickel can substitute for calcium in the binding with a membrane ligand such as the phosphate groups of a phospholipid in the process of nerve transmission and muscle excitation and contraction.

Perhaps the abnormality exists in the relationship between the membrane and DNA and/or RNA. Significant concentrations of nickel are present in DNA (33, 34) and RNA (9, 33, 35) from phylogenetically diverse sources. It has been suggested that nickel and the other metals which are present may contribute to stabilization of the structure of the nucleic acids. Nickel will stabilize RNA (36) and DNA (34) against thermal denaturation and is extraordinarily effective in the preservation of tobacco mosaic virus RNA infectivity (35, 37). Also, it has been reported that nickel may have a role in the preservation of the compact structure of ribosomes against thermal denaturation (38–40) and that nickel will restore the sedimentation characteristics of *Escherichia coli* ribosomes which have been subjected to EDTA denaturation. The preliminary findings on rat-liver polysomes, in addition to this *in vitro* evidence, suggest that nickel does have a structural role in nucleic acids.

In addition to possible roles in membrane and nucleic acid metabolism or structure, nickel has at least one structural role in proteins, that of nickeloplasmin (20). The function of this protein is unclear at present. It also should be noted that nickel can activate numerous enzymes *in vitro,* including arginase (41), tyrosinase (42), desoxyribonuclease (43), acetyl coenzyme A synthetase (44), and phosphoglucomutase (45). However, these studies have not shown nickel to be a specific activator of any enzyme.

SUMMARY

Nickel has met the criteria for essentiality. Recent research has shown that homeostatic mechanisms are present to regulate nickel metabolism. However, more studies are needed to ascertain whether ultrafiltrable organic nickel complexes are important in nickel homeostasis.

Nickel deficiency reproducibly results in an impairment of a function, or functions, as evidenced by the ultrastructural degeneration, reduced oxidative ability, and increased lipids and decreased phospholipid fractions in the liver of chicks. Rats deprived of nickel show a suboptimal reproductive performance, reduced oxidative ability in liver, and abnormalities in the liver polysome profile. These findings are consistent with nickel being an essential nutrient.

It is speculated that nickel has a structural or metabolic role in membrane, DNA, RNA, or protein biochemistry.

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DISCUSSION

Mills (Aberdeen). Do you have any information on the nickel content of liver subcellular fractions, particularly the mitochondria?

Nielsen. No, but we're working on it now. Sunderman has really developed the analytical technique, and he may have done some of these analyses.

Mills (Aberdeen). The ultrastructural changes that you are getting, including the pycnotic nuclei, are reminiscent of the type of change seen, not as a consequence of changes in the nuclear envelope, but as a change of oxygen tension in the cell. We've seen exactly the same sort of response while working with copper.

Schwarz (Long Beach). On the same issue, I might say that the electron microscopic changes which we see are very reminiscent of those which we described in 1956 and 1954 in the prenecrotic phase of dietary liver necrosis. You see a lack of oxygen consumption; do you also see a respiratory decline?

Nielsen. We have not studied this.

Panić (Zemun). Have you estimated the zinc content of tissues? We have found that the zinc content is changed in a nickel deficiency.

Nielsen. I have not determined the zinc content of the tissues.

Martin (Fort Collins). You mentioned nickel as an enzyme activator, and indicated that it is a nonspecific activator of arginase and tyrosinase. Is nickel more effective than other metal cations?

Nielsen. It is dependent on a number of factors. At certain pH's it is a better activator of arginase than other cations.

Armstrong (Minneapolis). How quickly is the deficiency produced by a nickel-deficient diet reversed on adding nickel to the diet?

Nielsen. I have not tried to reverse it. We find that some of the skin-pigmentation changes you see are evident at about 10 days on the deficient diet. It was also about the 10th to 14th day that we began to see differences in the oxidative ability of liver from deficient chicks.

Smith (Washington). Would you like to speculate on why serum nickel goes up during myocardial infarction, if indeed it does?

Nielsen. Yes, it does. Not only does it go up in certain diseases but it goes down in others, such as chronic urenia or liver cirrhosis. Yesterday, Dr. Burger reported a change in nickel during kwashiorkor.

Mertz (Beltsville). Did you say, Dr. Hennig, that you had produced a nickel deficiency?

Hennig (Jena). We think so, and we think that the pig has a higher nickel requirement than poultry. In the early 1960's we discussed an unidentified growth factor in a material that is a byproduct of vitamin C production. We have now determined the nickel content of this material, and see a close correlation between the growth of the pigs and the nickel content.

Nielsen. In our latest study, the rats from nickel-deficient dams do show about a 10% reduction in growth at 3½ weeks. At the present, I don't feel that this is significant.